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Research report

Neurochemical and behavioral deficits consequent to expression of a dominant negative EphA5 receptor

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Abstract

The Eph family tyrosine kinase receptors and their ligands have been linked to axon guidance and topographic mapping of the developing central nervous system. More specifically, the EphA5 receptor has been shown to play a role in development of hippocamposeptal, retinotectal and thalamocortical projections. Recently, a line of transgenic mice was developed which expresses a truncated EphA5 receptor lacking a functional tyrosine kinase domain. In a previous study, axonal tracing revealed that medial hippocampal axons in this strain projected laterally and ventrally away from their normal target area. In the current study, both transgenic and wild-type controls were evaluated in unconditioned (rotorod and locomotor activity) and conditioned (water maze and active avoidance) behavior tasks which tested hippocampal and striatal functioning. Compared to controls, the transgenic strain did not show differences in rotorod motor activity but did show a transient deficit in spatial navigation ability and a consistent impairment in active avoidance. The dominant-negative mutant receptor also resulted in a decrease in striatal dopamine and serotonin concentrations with no change in hippocampal monoamines. Collectively, these data suggest that animals expressing a truncated EphA5 receptor show deficits related to striatal functioning.

Theme: Neural basis of behavior
Topic: monoamines and behavior

Keywords: Transgenic; Eph; Behavior; Striatum; Mice

1. Introduction

The tyrosine kinase receptor, EphA5, and its ligand, ephrin-A5, have been implicated in the development of laminar circuits within the cortex [4,6,28,49] as well as development of the thalamocortical pathways [11]. The expression of EphA5 and its ligands shows a complementary pattern during development of these pathways and laminar circuits [4]. In addition, in vitro studies show that application of ephrin-A ligands to EphA5-expressing cells of the hippocampus and striatum results in reduction in neurite length and degeneration [2,10,11,13,14]. While the exact mechanism of this repulsion is not known, activation of EphA5 with ephrin-A5 results in F-actin dislocation from the leading edges back towards the center of the growth cone [3].

In addition, the role of EphA5 in development of retinotectal, entohrino-hippocampal and hippocamposeptal pathways has been demonstrated [7,40,48,50–53]. The medial to lateral gradient of EphA5 in the hippocampus reflects the topographic projection to the lateral septum in a chemorepulsive pattern with areas of high receptor expression (medial hippocampus) projecting to areas of low ligand concentration (ventrolateral septum) [52,53]. This suggests that the repellent interaction of EphA5 in the hippocampus with ligands in inappropriate afferent targets contributes to redirection of axons to appropriate target areas [9,40,52,53]. In addition to topographic map formation, the EphA5-ephrin-A chemorepulsive relationship may affect the function of hippocampal neurons. Application of a soluble ligand, which serves as an antagonist to the Eph receptor,

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impairs induction of long-term potentiation produced by tetanus stimulation [5,12,16]. Thus, the EphA5 receptor not only participates in developmental axon pathfinding, but may also mediate ongoing synaptic plasticity. In support of this conclusions, it was demonstrated that phosphorylation of the EphA5 receptor using a receptor agonist resulted in enhanced contextual fear conditioning while EphA5 blockade reduced alternation rate in a T-maze [15,16]. Both of these tasks have been shown to be highly sensitive to hippocampal function [15,16,20,25,36].

Finally, the EphA receptors have been topographically localized to different striatal compartments [22]. Specifically, EphA4 and EphA7 and their ligands (ephrin-A4 and ephrin A2, A3) were found to be restricted to the matrix and striosome areas within the striatum, respectively [22,30]. In addition, it has been reported that EphA5 is found in the substantia nigra and neostriatum of the mouse [1,47] and localized on the cell surface of limbic areas and basal ganglia of the human brain [33,34].

In order to examine the effects of alteration of EphA receptors in mice, a transgenic strain was generated which expressed a truncated tyrosine kinase domain of the EphA5 receptor. It has been demonstrated that this mutant receptor inhibited activation of the EphA3 and EphA5 receptors and disrupted normal projection of the medial hippocampal neurons to the lateral septum [48]. This is consistent with results from an ephrin-A5 or ephrin-A5/ephrin-A2 homozygous null mutant, which results in an "overshoot" of retinal projections to inferior colliculus [46]. The aim of the current study was to determine the functional contribution of EphA receptors to behavioral function and neural plasticity. The effects of EphA receptor inhibition on drug-induced behavior were analyzed using dopaminergic agonists, amphetamine and cocaine. Finally, regional brain levels of monoamines were measured in an attempt to link the behavioral responses of these mice to possible neurochemical alterations.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. For rotorod and horizontal activity experiments, 18 transgenic mice ($n=13$ male and 5 female) and 10 control mice ($n=4$ male and 6 female) were used. For water maze and two-way active avoidance training, a new set of experimentally and drug naive male mice (both transgenic ($n=12$) and wild-type ($n=8$)) were used. All mice were at least 5 months old at the time of first behavioral testing. Animals were housed in a colony room with a 12 h on/12 h off light cycle. The transgenic mice were generated according to the methods described previously [48]. Briefly, a purified transgene product was microinjected into fertilized F1 mouse eggs on a C57BL/6J and C2H/HeJ background. This transgene was demonstrated to inhibit wild-type activation of the EphA5 receptor by disrupting the tyrosine kinase domain [48]. Tail DNA from mice born to injected embryos was screened for the transgene using Southern Blot Analysis. A green fluorescence

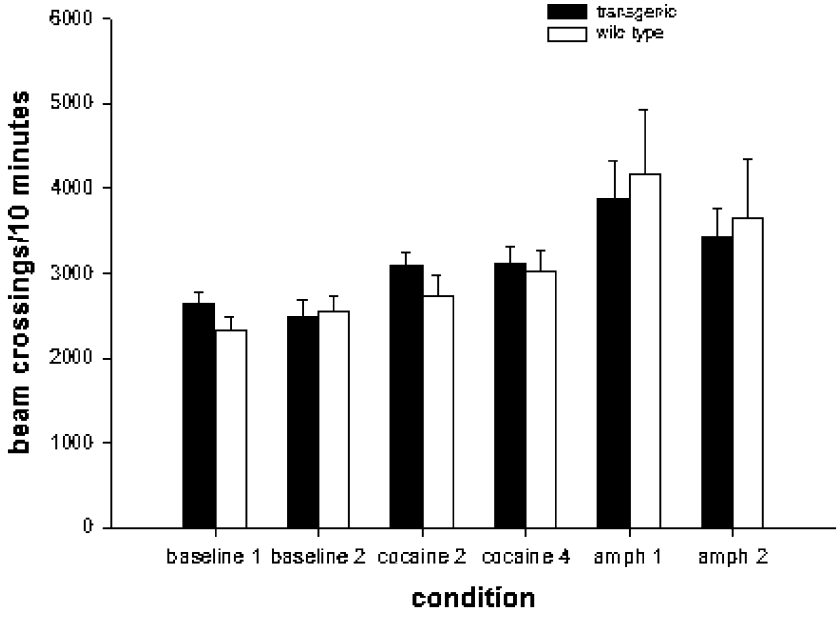

Fig. 1. Motor activity in EphA5 transgenic and wild-type mice. Animals were tested for two 10-min baseline periods and tested following the administration of 2 and 4 mg/kg cocaine and 1 and 2 mg/kg amphetamine. Both amphetamine doses significantly increased motor activity; however, there was no difference between strains.

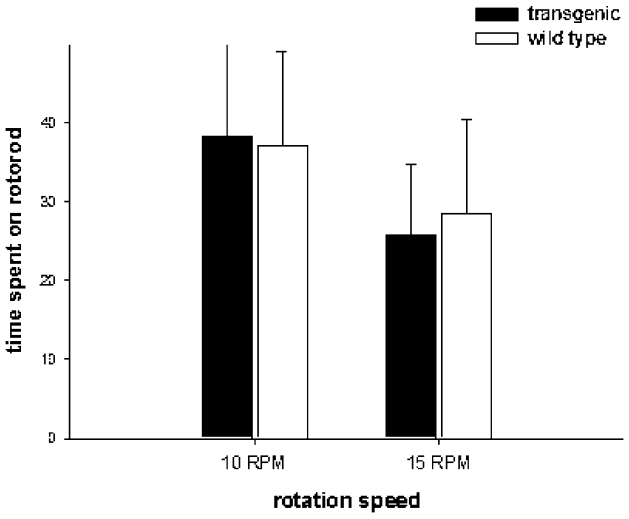

Fig. 2. Rotorod performance in EphA5 transgenic and wild-type mice. Both strains of mice were tested on the rotorod at 10 and 15 rotations per min (RPM) and allowed a maximum of 120 s on the rod. There was no significant effect of genotype on latency to fall.

probe attached to the Bam H1 site downstream of the EphA5 transmembrane domain was present in only mice which expressed the injected transgene.

2.2. Locomotor activity

Horizontal motor activity was measured using an Opto-Varimex-Minor Instrument (Columbus Instruments, Columbus, OH). Animals were placed inside the activity chamber measuring 42.5×40 cm for 10 min. Twelve horizontal infrared beams were placed 2.5 cm apart and approximately 1.25 cm from the bottom of the chamber. Total horizontal activity was recorded as any intersection of beams on the X or Y axis. Animals were given two drug-free (baseline) sessions spaced 4 days apart. One week following the second baseline session, animals were injected IP with 2 or 4 mg/kg cocaine hydrochloride or 1 or 2 mg/kg amphetamine sulfate (Sigma, St. Louis, MO) and tested 20 min following cocaine administration and 15 min following amphetamine administration. Each drug administration period was spaced at least 1 week apart, and drugs were administered in a pseudo-latin square design.

2.3. Rotorod

The rotorod (13 cm circumference) was rotated at a rate of 10 or 15 rotations per min (RPM). Animals were placed on the rod while it was rotating and time spent on the rod was recorded. Each animal was allowed a maximum of 180 s. Only the second trial was counted at each speed. A box filled with wood chip bedding served as a cushion and was located 130 cm beneath the rod.

2.4. Water maze

A circular galvanized steel tub 61 cm in diameter and 29 cm in height was filled 3/4 full with room-temperature water and made opaque with nontoxic white latex paint. A circular escape platform, measuring 18 cm in diameter, was placed in one quadrant of the maze such that the top was 4 cm below the surface of the water. Animals were removed from the home cage and allowed a 60-s habitu-

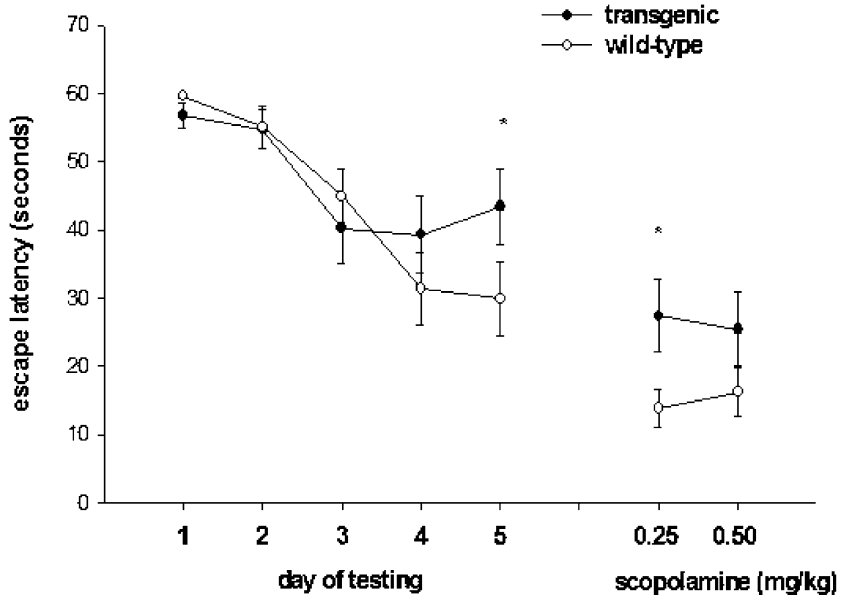

Fig. 3. Water maze performance in EphA5 transgenic and wild-type mice. Both groups were trained for 5 days in a circular water maze with a submerged platform. Following the acquisition phase, animals were not tested for 14 days, then retested for 5 days. *Indicates significantly different from wild-type mice, $p < 0.05$, using Fisher's PLSD.

ation in the maze prior to testing. Following habituation, animals were given four trials per day, each trial starting at a different quadrant of the circular maze. Each starting point was equidistant apart, and randomly selected on each day of testing. The subjects were allowed a maximal time of 60 s per trial. If the subject did not find the hidden platform within 60 s, the animal was placed on the platform for a period of 30 s before starting the next trial. All subjects were run for five consecutive days and then not trained for a 14-day retention period, after which they were run for five more consecutive days. This allowed both assessment of acquisition of the task and long-term retention. The position of the platform remained constant during testing. Following retention training, all animals were administered the acetylcholine muscarinic antagonist scopolamine hydrobromide (Sigma) IP at doses of 0.25 and 0.50 mg/kg (spaced 2 days apart) 30 min prior to testing for 1 day. Scopolamine has been shown to impair acquisition of spatial navigation learning but has minimal effects on retention [37].

2.5. Two-way active avoidance

The avoidance chamber consisted of a Plexiglass shuttle box measuring 27 cm in length, 10.7 cm wide and 16.8 cm high. The floor was made of stainless steel bars with a 0.75 cm space between each. When the subject moved past the center of the chamber on either side, the weight of the animal shifted the angle of the floor by 1.5 cm. A 5 cm speaker which generated a tone [serving as the conditioned stimulus (CS)] was mounted on each side. The shuttle box was housed inside an outer sound-attenuating chamber illuminated by a 6 W bulb for the entire session.

Animals were placed on one side of the shuttle box at the start of each session. Following a 10 s tone CS, animals received a 10 s maximum shock US (1 mA scrambled footshock) on that side of the chamber. The correct response, moving to the opposite side during the tone, terminated the tone and avoided the shock. Escape responses during the shock terminated tone and shock. The parameters recorded were the number of avoidances

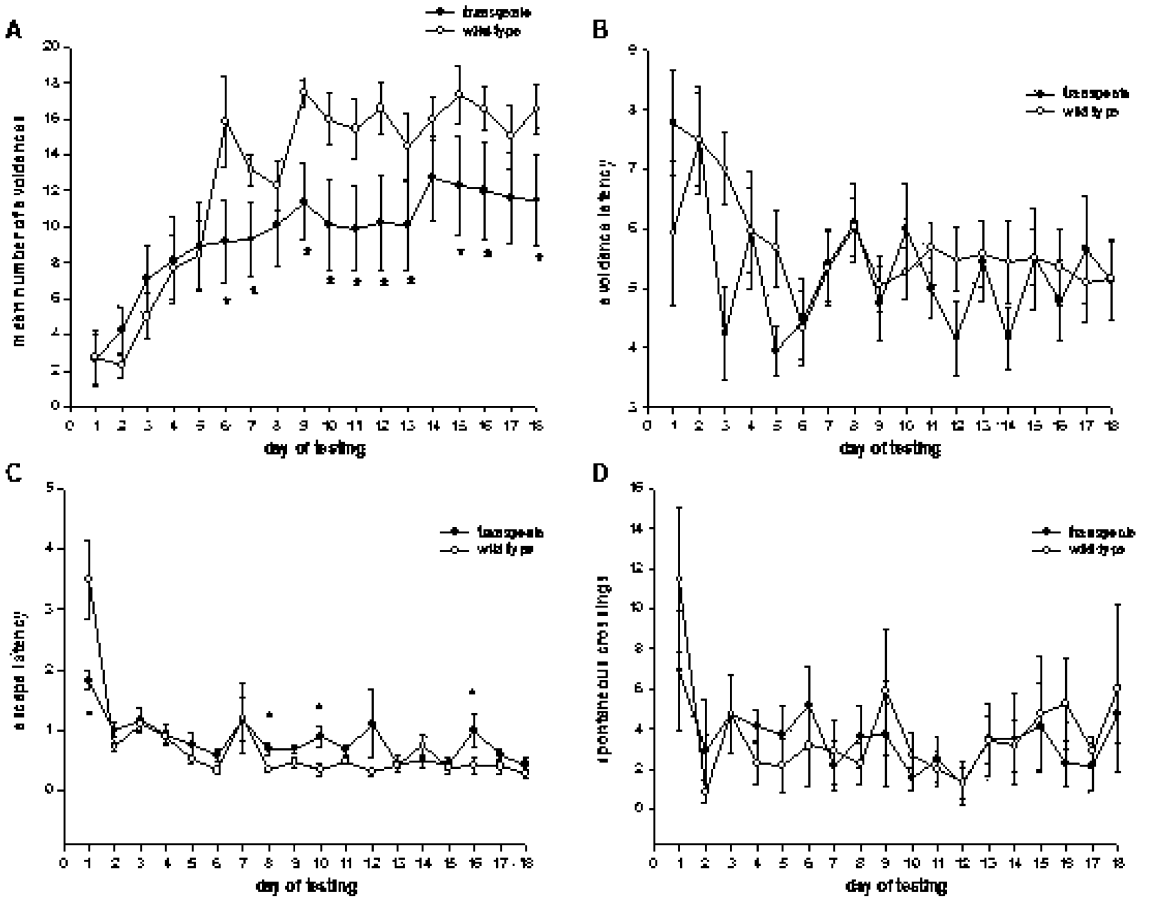

Fig. 4. Two-way active avoidance in EphA5 transgenic and wild-type mice. Panel A: mean number of avoidances in a 20 trial two-way active avoidance procedure over an 18-day testing period. Panel B: mean avoidance latency (calculated out of number of avoidances) in a 20 trial two-way active avoidance procedure over an 18-day testing period. Panel C: escape latency (calculated from number of escapes) in a 20 trial two-way active avoidance procedure over an 18-day testing period. Panel D: spontaneous crossings during the 10 s intertrial interval in a 20 trial two-way active avoidance procedure over an 18-day testing period. *Indicates significantly different from wild-type mice, $p < 0.05$, Fisher's PLSD.

out of 20 total trials, avoidance latency (calculated out of total number of avoidances), escape number, escape latency (calculated out of total number of escapes) and spontaneous crossings between the two sides of the shuttle box during the 20 s intertrial interval.

2.6. Neurochemical analysis

Forty-eight hours following the last session, all animals were sacrificed and brains removed for neurochemical assay. The frontal cortex, striatum and hippocampus were dissected on a cold block and stored in liquid nitrogen until assay using HPLC [18].

3. Results

3.1. Locomotor activity

Under baseline conditions, mice had an average of 2200 counts/10 min period, with no significant differences between strain or sex. Both doses of amphetamine (1 and 2 mg/kg) significantly increased motor activity in both groups of mice ($F(5, 125) = 7$, $p = 0.0001$) (Fig. 1). Cocaine, at these doses, did not alter behavior nor was there any difference between the two strains on any drug-free or drug-treatment day.

3.2. Rotorod

At 10 RPM, both groups were able to spend approximately 36 s on the rotating cylinder. At 15 RPM, animals showed a slight decrease in time spent on the rod ($F(1, 26) = 3.9$, $p = 0.05$). However, there was no difference between groups at either speed (Fig. 2).

3.3. Water maze

On the first day of training, both wild-type and transgenic mice spent close to the full 60 s time limit in searching for the hidden escape platform. However, by day 3, performance improved such that both groups showed a significant decrease in escape latency ($F(10, 198) = 3316.6$, $p = 0.0001$). A significant interaction revealed a significant increase in escape latency in transgenic animals on day 5 of training as well as following 0.25 mg/kg scopolamine (a cholinergic antagonist) ($F(10, 198) = 1.9$, $p = 0.03$) as compared to wild-type controls (Fig. 3). When animals were retrained 14 days after the acquisition phase, there were no significant differences in performance between strains (data not shown).

3.4. Active avoidance

Both groups showed poor avoidance responding on the first few days of training; however, over sessions ($F(17, 255) = 18.1$, $p = 0.0001$) mice showed an improvement in performance. By day 6, the control group was avoiding at a rate of approximately 80%, whereas the transgenic mice were still avoiding the shock less than 50% of the trials ($F(17, 255) = 3$, $p = 0.0002$) (Fig. 4A). For the rest of training, with the exception of days 8 and 14, the transgenic mice were unable to achieve avoidance responding to the same level as the wild-type controls. Both groups showed a significant decrease in avoidance latency over time ($F(17, 255) = 3.2$, $p = 0.0001$) and the two groups were not different from each other on this measure (Fig. 4B). Furthermore, both the transgenic and wild-type mice demonstrated a significant decrease in escape latency following only 1 day of training ($F(17, 238) = 11.7$, $p = 0.0001$). On the first day of training, the control animals were significantly slower to escape than the transgenics ($F(17, 238) = 3$,

Table 1
Neurotransmitter and metabolite levels in brain regions of transgenic ($n = 10$) and wild-type ($n = 7$) animals used in water maze and two-way avoidance experiments

Striatum

Genotype	Dopamine	DOPAC	5-HT	5-HIAA	HVA
Transgenic	9.91 (0.905)*	0.755 (0.113)*	0.565 (0.039)*	0.322 (0.024)*	1.55 (0.176)
Wild-type	17.82 (3.26)	1.341 (0.195)	0.842 (0.078)	0.496 (0.082)	2.45 (0.591)

Hippocampus

Genotype	Dopamine	5-HT	5-HIAA
Transgenic	0.087 (0.031)	0.980 (0.071)	0.596 (0.042)
Wild-type	0.064 (0.021)	1.101 (0.187)	0.658 (0.093)

Frontal cortex

Genotype	Dopamine	DOPAC	5-HT	5-HIAA
Transgenic	1.01 (0.228)	0.175 (0.033)	0.752 (0.070)	0.106 (0.017)
Wild-type	1.02 (0.258)	0.143 (0.029)	0.686 (0.076)	0.164 (0.032)

Values expressed as μg/g wet tissue. Numbers in parenthesis represent standard error of the mean.
* Indicates significantly different from wild-type controls using independent samples t-test with $p < 0.05$.

$p = 0.0001$), but by day 8, transgenic animals were slower to escape than controls (Fig. 4C). This increase in escape latency was present for the remainder of the study, reaching statistical significance on days 8, 10, 12 and 16. Furthermore, spontaneous crossings between chamber sides during the intertrial interval decreased over days ($F(17, 221) = 2$, $p = 0.0089$) (Fig. 4D). This decrease is indicative of a habituation effect, an effect that did not differ between the two groups tested.

3.5. Neurochemistry

Transgenic mice exhibited significantly lower levels of striatal dopamine ($t(15) = -2.7$, $p = 0.02$), 3,4-dihydroxyphenylacetic acid (DOPAC) ($t(14) = -2.1$, $p = .04$), serotonin (5-HT) ($t(15) = -3.44$, $p = 0.004$) and 5-hydroxyindoleacetic acid (5-HIAA) ($t(15) = -2.4$, $p = 0.03$) compared to wild-type animals. No differences in any transmitter or transmitter metabolite was detected in the frontal cortex or hippocampus (Table 1).

4. Discussion

The results of the current experiments show transgenic expression of a dominant-negative mutant EphA5 receptor produces impairments in two-way active avoidance learning with concomitant monoamine depletion in the striatum. These animals showed a significant decrease in avoidance number starting around day 6 of training, most likely due to a significant increase in avoidance number in wild-type controls after this day. The transgenic mice also showed a significant increase in escape latency compared to wild-type controls even though they were initially faster to escape the shock. Since intertrial crossings and rotorod performance were unchanged compared to wild-type controls, it is unlikely that the deficits in avoidance and escape responding were due to a generalized motor deficit. Finally, these mice showed only a transient deficit in spatial water maze performance compared to wild-type animals and did not show differential sensitivity to the dopaminergic agonists, amphetamine or cocaine.

The specific impairment in acquisition of a two-way avoidance paradigm is consistent with those reported following striatal dopaminergic deficits; indeed, impaired striatal function as assessed by the active avoidance paradigm is considered to be the classic model of Parkinson's disease as well as of dopamine receptor blockade associated with antipsychotic therapy [8,19,21,26,27,35,44,45]. Together with the specific decrease in striatal dopamine and serotonin concentrations, it may be concluded that the behavioral deficits observed in these transgenic mice may be consequent to compromised striatal functioning. At this time, it is not clear why these transgenic mice exhibit altered levels of these monoamineregic transmitters. Possible explanations might include alterations in the neurodevelopment of the dopaminergic projections to the striatum from the mesencephalon or modifications in synaptic function or integrity in the adult mice. Nonetheless, the behavior deficits and reduction in striatal dopamine observed in EphA5 dominant negative receptor mice do suggest that EphA receptors play important roles in the functions of dopaminergic neurons. Consistent with this proposal, EphA5 is expressed in the midbrain dopaminergic neurons [29]. In addition, ephrin-A5, a ligand of EphA receptors, is transcribed in the striatum (R. Zhou, unpublished observations). The presence of EphA5 and ephrin-A5 in dopaminergic neurons and targets, respectively, suggests that this receptor-ligand pair participates in the regulation of substantia nigra–striatum interactions.

While the dominant negative mutant EphA5 receptor produces a shift of hippocampal neurons to the ventral lateral septum, the deficit in active avoidance is unlikely to be the result of alterations in septal mapping, as lesions to the septum facilitated, rather than impaired, two-way avoidance performance [24]. Interestingly, animals which expressed a dominant negative EphA5 receptor showed only a transient increase in escape latency on the water maze. Previous lesion studies have reported that destruction of the hippocampus or the major output of the hippocampus, the fimbria/fornix, disrupts performance on the water maze [32,41,42]. Thus, if functioning of the entire hippocampus in these animals was compromised, a deficit in water maze acquisition would be expected. However, in the transgenic mice, only the neurons from the medial, but not the lateral hippocampus, showed a "ventral shift" in projections [48]. Therefore, it is possible that the displacement of these axons from the hippocampus are not solely responsible for the acquisition of spatial navigation behaviors in the mouse and that hippocampal function is only mildly affected in these mice. While the hippocampus is thought to modulate associations between different stimuli and the relationship of the stimuli to the reinforcer, it is not essential for forming simple stimulus-response associations [31] such as the two-way active avoidance. Few studies have reported deficits in two-way active avoidance without a spatial component following hippocampal lesions. As striatal lesions have produced errors in spatial navigation [38], the transient deficit in water maze may possibly be linked to dysfunction of the striatum and not the hippocampus.

Another possible theory to account for the mild disruption of hippocampally mediated behaviors is that projections to the striatum via the fimbria/fornix and entorhinal cortex were disrupted [17,23,39,43]. It should be noted that the transgenic strain was differentially sensitive to a low dose of scopolamine when administered after both groups had learned the task, suggesting that the EphA5 mutation resulted in an alteration in cholinergic receptor sensitivity.

In summary, animals with a transgene that disrupted function of the EphA tyrosine kinase receptors displayed specific deficits in active avoidance and a decrease in monoamine concentrations in the striatum. These mice did

not show severe deficits in water maze performance although they were sensitive to the disruptive effects of scopolamine. The EphA5 receptor has been reported to be critical for both developmental axon projection signaling, and ongoing synaptic plasticity. It is likely that the behavioral deficits seen in mice possessing a dominant-negative mutant EphA5 receptor reflect deficits in both of these functions.

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